

**UNIVERSITY RESEARCH GRANT  
FINAL REPORT**  
*Geran Penyelidikan Universiti  
Laporan Akhir*

<b>A.</b>	<b>PARTICULARS OF RESEARCH / MAKLUMAT PENYELIDIKAN:</b>
(i)	<b>Title of Research:</b> <i>Tajuk Penyelidikan:</i>  <b>Molecular and Immunological Profiling of Typhoid Carriers and Salmonella Typhi: Towards Development of Preventive and Investigative diagnostics</b>
(ii)	<b>Account Number:</b> <i>Nombor Akaun:</i> <b>1001/CIPPM/8130131</b>
<b>B.</b>	<b>PERSONAL PARTICULARS OF RESEARCHER / MAKLUMAT PENYELIDIK:</b>
(i)	<b>Name of Research Leader:</b> <i>Nama Ketua Penyelidik:</i>  <b>Profesor Asma Ismail</b>

**Name of Co-Researcher**

*Nama Penyelidik Bersama:*

**Sub project 1: Investigative culture and PCR test studies**

Lead : Prof Asma Ismail

Dr Lila P. Mohamed Meeran -MOH

Dr Hani Mat Hussain -MOH

Dr Sharina Dir -MOH

Dr. Aziah Ismail

Dr Kirmpal Kaur

Siti Norazura Mohamad

Amy Amilda Anthony

Elis Rosliza Mohd Adzmi

Prof M. Ravichandran (AIMST)

Prof John Wain- Health Protection Agency

Dr Satheesh, Health Protection Agency

Prof Ataharul, Univ of Dhaka

**Sub-project 2: Immunological profiling of host**

Lead : Prof Prabha Balaram

AP Phua Kia Kien

Dr Aziah Ismail

B Saatheeyavaane

Nur Eliyana

**Sub-project 3: Antigenic profile of carriers**

Lead : Prof Asma Ismail

Dr Kirmpal Kaur

Dr Rohman Naim

Amy Amilda Anthony

**Sub-project 4: DNA profiling****a. Molecular epidemiology**

Lead : AP Phua Kia Kien

Prof Thong Kwai Lin

Prof Prabha Balaram

Nor Fadhilah Kamaruzzaman

**b. Genome sequencing studies**

Lead: Dr Aziah Ismail

AP Phua Kia Kien

Dr Venugopal Balkrishnan

Dr Satheesh Nair

**Sub - project 5: Design and development of diagnostics**

Lead :Prof Asma Ismail

Amy Amilda Anthony

Dr Aziah Ismail

Dr Kirmpal Kaur

Dr. Sugumar Dharmalingam

AP Ling Xue Kong

**Sub-project: 6 Evaluation of tests**

Lead : AP Habsah Hassan

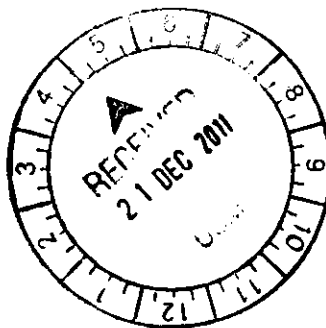
Dr Kirmpal Kaur

Dr S. Niyogi

Dr Lila P. Mohamed Meeran

Dr Hani Mat Hussain

Dr Sharina Dir





## **F. SUMMARY OF RESEARCH FINDINGS**

*Ringkasan dapatan Projek Penyelidikan*

### **Significant findings**

- The improved stool sampling method (fixed weight sampling method) was successful in detecting typhoid carriers in Kelantan. We were successful in developing the first carrier registry for Kelantan as well as Malaysia.
- The PCR assay could match the culture results suggesting our PCR test could be an alternative method to detect for carriers. For antimicrobial sensitivity test, the culture still needs to detect for antimicrobial sensitivity.
- When screened for biomarkers, the carrier IgG and IgA showed that it recognized only the 50Kda antigen of S.Typhi. The *TYPHIDOT-c* was shown to be a potential serological screening tool to detect for possible carriers. Those positive can be further analysed for culture and PCR. This would be a more cost-effective approach rather than culture method alone. The success of detecting typhoid carriers will create an impact to public health. The *TYPHIDOT-c* assay can be used to screen for typhoid carriers among food handlers and immigrants and further confirmed by culture and PCR. An improved Typhidot in the form of a dipstick would be better suited for the field .
- Established the first genome sequence bank of S.Typhi that are isolated from confirmed carriers from Malaysia (MyTyphoidgenom) The specific sites could not be considered of value as epidemiological markers as well as diagnostic purposes since they are no unique sequences for S. Typhi isolated from typhoid carriers.
- Sequence analysis showed that typhoid carriage is Not due to the organism but rather due to host factors
- Immunological profiling of healthy adults and typhoid carriers showed that reduced levels of TH1 and TH2 cytokines might suggest a carrier status
- The candidate gene (2136 bp Polynucleotide phosphorylase gene) was used to test if typhoid carrier state in humans was due to a mutation in this gene that was reported to determine virulence and carrier status of *S.typhimurium* in mice. Results showed that there were 11 silent mutations in the PNPase gene (hence did not affect protein expression). Hence the findings reported for *S.typhimurium* in mice cannot be extrapolated to S.Typhi that affect only humans
- In developing the dipstick method for Antibody carrier detection, we have shown by POC that the dipstick method is working in our laboratory for detection of IgA. When outsourced for the cassette format for both IgG and IgA requires more time for fine tuning.



**G. COMPREHENSIVE TECHNICAL REPORT**

*Laporan Teknikal Lengkap*

Applicants are required to prepare a comprehensive technical report explaining the project.  
(This report must be attached separately)

Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.  
[Laporan ini mesti dikepilkan]

A comprehensive and detailed technical report of the grant is as explained in the powerpoint attached in Appendix 2.

**List the key words that reflect your research:**

*Senaraikan kata kunci yang mencerminkan penyelidikan anda:*

English	Bahasa Malaysia
Typhoid carriers	Pembawa tifoid
Salmonella Typhi	Salmonella Typhi
Carrier diagnostics	Diagnostik pembawa

H. a) Results/Benefits of this research  
Hasil Penyelidikan

No. Bil:	Category/Number: Kategori/ Bilangan:	Promised	Achieved
1.	<b>Research Publications (Specify target journals)</b> <i>Penerbitan Penyelidikan (Nyatakan sasaran jurnal)</i>	14	ISI journals = 5 Supplement = 3 Abstract in conf proceedings = 12 Total: 20
2.	<b>Human Capital Development</b>		
	a. Ph. D Students	6	4
	b. Masters Students		2
	c. Undergraduates (Final Year Project)		-
	d. Research Officers		-
	e. Research Assistants		9
	f. Other: Please specify (Science officers, Senior MLT, MLT)		6
3.	<b>Patents attained</b> <i>Paten</i>	3	3
4.	<b>Specific / Potential Applications</b> <i>Spesifik/Potensi aplikasin</i>	6	6
5.	<b>Networking &amp; Linkages</b> <i>Jaringan &amp; Jalinan</i>	9	10
6.	<b>Possible External Research Grants to be Acquired</b> <i>Jangkaan Geran Penyelidikan Luar Diperoleh</i>	2	1

- Kindly provide copies/evidence for Category 1 to 6.

b) Equipment used for this research.  
Peralatan yang telah digunakan dalam penyelidikan ini.

Items Perkara	Approved Equipment	Approved Requested Equipment	Location
<b>Specialized Equipment</b> Peralatan khusus	2 x POWER PAC 2 x ELECTRO-ELUTER 2 x MANUAL MULTIPETTE PLUS	1. Balance (2 decimal point) 2. CLCbio Genomic Workbench software, Perpetual License 3. DNA electrophoresis system with power pack 4. High End Computer 5. Microcentrifuge with adapter for 0.2 ml 1.5 ml 6. Nephelometer 7. SDS-PAGE with 6 gel capacity with casting chamber 8. Power pack for SDS PAGE 9. Belly Dancer 10. High Capacity Power Supply 11. Incubator 12. Innova Upright Ultra-Low Temp – Freezer 13. Microcentrifuge With Rotor 14. Microcentrifuge 15. Sds-Page Gel Electrophoresis 16. Semi-Dry Electrophoretic Transfer Cell 17. Thermal Cycler With Gradient Option 18. Vortex Mixer	INFORMM
<b>Facility Kemudahan</b>	2 x PRINTER 2 x COMPUTER 2 x LAPTOP 2 x SCANNER 1 x PRINTER COLOUR	1. 1 x Computer	INFORMM
<b>Infrastructure</b> Infrastruktur	Infrastructure Research & Innovation – Universiti Sains Malaysia – 2011331		

Please attach appendix if necessary.

I. **BUDGET / BAJET**

**Total Approved Budget** : RM 333,333.34  
**Total Additional Budget** : RM 1,500,000.00  
**Grand Total of Approved Budget** : RM 1,833,333.34

**Yearly Budget Distributed**

Year 1 : RM 116,300.00  
Year 2 : RM 217,033.34  
Year 3 : RM 1,500,000.00

**Additional Budget Approved**

Year 1 : RM  
Year 2 : RM 1,000,000.00  
Year 3 : RM 500,000.00

**Total Expenditure** : RM 1,831,462.92  
**Balance** : RM 1,870.42

- Please attach final account statement from Treasury

*b/p Chial*

**Signature of Researcher**

Tandatangan Penyelidik

**PROFESOR ASMA ISMAIL**

Penyeras Geran

Molecular and Immunological Profiling of Typhoid  
Carriers and Salmonella Typhi: Towards Development  
of Preventive and Investigative  
( 1001/CIPPM/8130131 )

*20/12/2011*

**Date**

Tarikh





# FINANCIAL STATUS

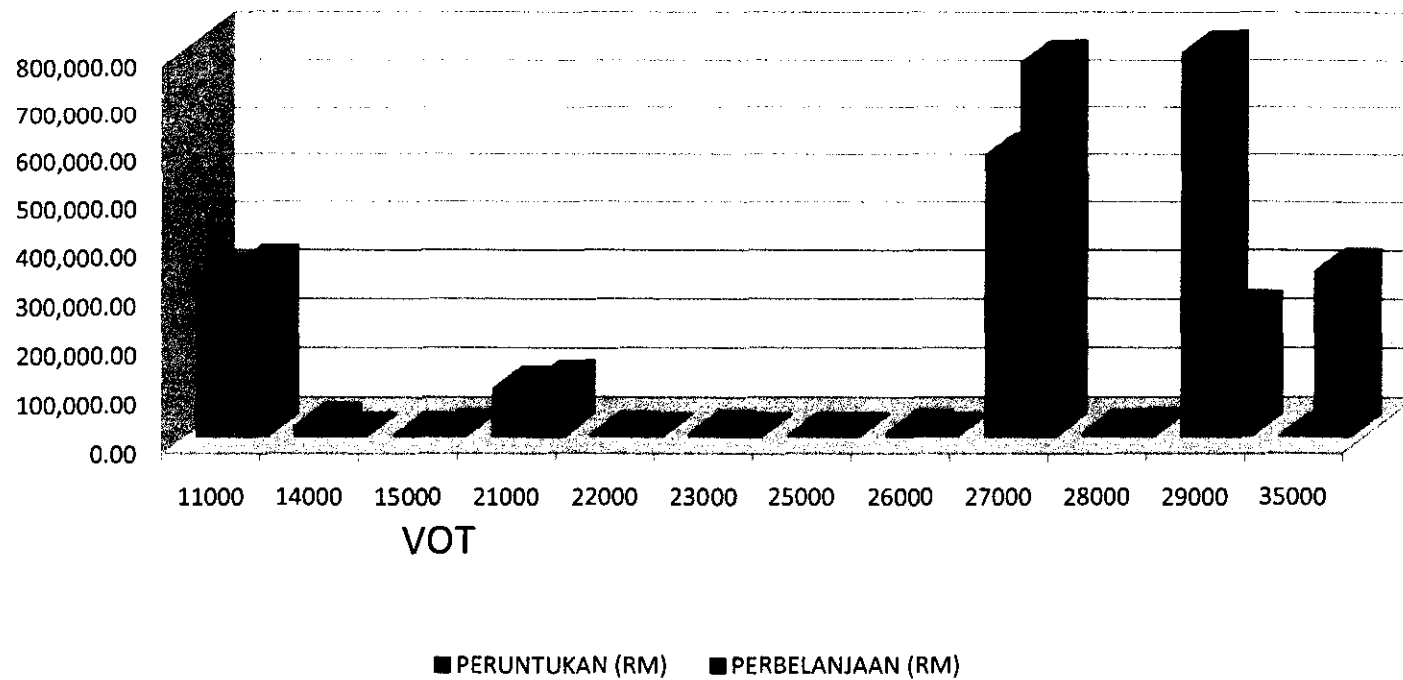
# Financial Expenditure

Amount of Grant Approved	:	RM 333,333.34
Total grant approved	:	RM 1,833,333.34
Cumulative Disbursement (07/07/2009)	:	RM 1,333,333.34
Additional Allocation OKT 09 = RM 1,000,000.00	:	
<del>Balance of Allocation (already received)</del>	:	RM 500,000.00

No.	Items	Approved Grant (RM)	Expenditure to date	
			Actual Spending (RM)	Commitment (RM)
99.89% expenditure				
1.	Temporary and Contract Personnel (V11000)	325,800.00	307,824.59	34,818.29
2.	(V14000)	20,000.00	1,478.93	1,265.69
3.	(V15000)	-	5,000.00	1,500.00
4.	Travel expenses and subsistence(V21000)	96,000.00	72,214.59	33,055.50
5.	Transportation of goods(V22000)	2,000.00	-	2,000.00
6.	Communications and utilities (V23000)	4,600.00	626.67	562.67
7.	(V25000)	-	636.20	149.50
7.	Supply of materials for repair and maintenance (V26000)	6,000.00	172.50	-
8.	Research Materials and Supplies (V27000)	577,733.34	619,017.09	156,569.14
9.	Minor Modifications and Repairs (V28000)	6,000.00	-	11,160.00
10.	Special Services (V29000)	795,200.00	233,786.43	14,591.13
11.	Special Equipment and Accessories (V35000)	-	19,458.00	317,576.00
TOTAL (RM)		1,833,333.34	1,260,215.00	571,247.92

Balance RM 1,870.02

**GRAF PERBELANJAAN GERAN 1001/CIPPM/8130131 HINGGA 18 DISEMBER 2011  
- PROFESOR ASMA ISMAIL**



VOT	PERUNTUKAN (RM)	PERBELANJAAN (RM)	BAKI (RM)	PERATUS %
11000	325,800.00	342,642.88	-16,842.88	105.2
14000	20,000.00	1,265.69	18,734.31	6.3
15000	0	6,500.00	-6,500.00	0
21000	96,000.00	106,749.02	-10,749.02	111.2
22000	2,000.00	0	2,000.00	0
23000	4,600.00	1,189.34	3,410.66	25.9
25000	0	785.70	-785.70	0
26000	6,000.00	172.50	5,827.50	2.9
27000	577,733.34	775,586.23	-197,852.89	134.2
28000	6,000.00	11,160.00	-5,160.00	186
29000	795,200.00	248,377.56	546,822.44	31.2
35000	0	337,034.00	-337,034.00	0
<b>JUMLAH</b>	<b>1,833,333.34</b>	<b>1,831,462.92</b>	<b>1,870.42</b>	<b>99.9</b>

UserCode: IZZAT / USMKCKLIVE / CIPPM

Program Code: Votebook9100

Current Program : Votebook (Header)

Current Date : 18/12/2011 9:24:45 PM

Version: 15.01, Last Updated at 19/10/2011

DB: 13.00, 9/18/2010 VB: 13.01, 3/14/2011

Switch Language : English / Malay

Wildcard : eg. Like 100%, Like 10%1, Like %1

Element 1: 1001

Element 2: %

Element 4: CIPPM

Element 5: 8130131

Year: 2011

Group:

Summary Total

Detail Excel	Budget Rule	Budget Control	Account Description	Budget Account Code	Roll over	Budget	Cash Received	Advanced	Commit	Actual	Available	Percentage
Detail Excel	46	T	Projek Kumpulan Wang Uni Penyelidikan	1001.111.0.CIPPM.8130131	0.00	17,975.41	0.00	0.00	0.00	34,818.29	-16,842.88	-93.70%
Detail Excel	46	T	Projek Kumpulan Wang Uni Penyelidikan	1001.114.0.CIPPM.8130131	0.00	18,521.07	0.00	0.00	0.00	1,265.69	17,255.38	93.17%
Detail Excel	46	T	Projek Kumpulan Wang Uni Penyelidikan	1001.115.0.CIPPM.8130131	0.00	-5,000.00	0.00	0.00	0.00	1,500.00	-6,500.00	130.00%
	46	T	SubTotal		0.00	31,496.48	0.00	0.00	0.00	37,583.98	-6,087.50	-19.33%
Detail Excel	47	T	Projek Kumpulan Wang Uni Penyelidikan	1001.221.0.CIPPM.8130131	0.00	23,785.41	0.00	0.00	1,545.00	31,510.50	-9,270.09	-38.97%
Detail Excel	47	T	Projek Kumpulan Wang Uni Penyelidikan	1001.222.0.CIPPM.8130131	0.00	2,000.00	0.00	0.00	0.00	0.00	2,000.00	100.00%
Detail Excel	47	T	Projek Kumpulan Wang Uni Penyelidikan	1001.223.0.CIPPM.8130131	0.00	3,973.33	0.00	0.00	67.90	494.77	3,410.66	85.84%
Detail Excel	47	T	Projek Kumpulan Wang Uni Penyelidikan	1001.225.0.CIPPM.8130131	0.00	-636.20	0.00	0.00	0.00	149.50	-785.70	123.50%
Detail Excel	47	T	Projek Kumpulan Wang Uni Penyelidikan	1001.226.0.CIPPM.8130131	0.00	5,827.50	0.00	0.00	0.00	0.00	5,827.50	100.00%
Detail Excel	47	T	Projek Kumpulan Wang Uni Penyelidikan	1001.227.0.CIPPM.8130131	0.00	-41,283.75	0.00	0.00	4,051.00	152,518.14	-197,852.89	479.25%
Detail Excel	47	T	Projek Kumpulan Wang Uni Penyelidikan	1001.228.0.CIPPM.8130131	0.00	6,000.00	0.00	0.00	4,160.00	7,000.00	-5,160.00	-86.00%
Detail Excel	47	T	Projek Kumpulan Wang Uni Penyelidikan	1001.229.0.CIPPM.8130131	0.00	561,413.57	0.00	0.00	900.00	13,691.13	546,822.44	97.40%
	47	T	SubTotal		0.00	561,079.86	0.00	0.00	10,723.90	205,364.04	344,991.92	61.49%
Detail Excel	48	T	Projek Kumpulan Wang Uni Penyelidikan	1001.335.0.CIPPM.8130131	0.00	-19,458.00	0.00	0.00	242,318.00	75,258.00	-337,034.00	1,732.11%
	48	T	SubTotal		0.00	-19,458.00	0.00	0.00	242,318.00	75,258.00	-337,034.00	1,732.11%
	9999		GrandTotal		0.00	573,118.34	0.00	0.00	253,041.90	318,206.02	1,870.42	0.33%

# **Appendix 1**



# Appendix 1

## ABSTRACT

Detection of typhoid carriers is not easy due to the lack of effective lab tests for carriers. If we can design diagnostic tests to detect for carriers, we can provide treatment, create a carrier registry and study the carriers himself/herself – (Fundamental and clinical studies), isolate *S.Typhi* from the carriers, sequence its DNA and compare that to those isolated from acute cases – (Molecular studies). In fact many studies (goldmine) can be done if we can detect for carriers.

In this proposal we undertake to study individuals who were previously culture positive for *Salmonella Typhi* for a minimum of 1 year with respect to the following objectives:

- To develop new methods to identify possible carriers
- To determine the OMP antigens that are antigenically specific to typhoid carriers
- To determine the immunological factors that predispose individuals to become carriers
- To sequence and compare the DNA profiles of *S. Typhi* isolated from acute cases, carrier cases, the environment and the *S. Typhi* DNA sequence (gene bank)
- To develop diagnostics for detection of typhoid carriers
- Perform evaluation of the developed tests

Among the achievements of studies include:

Establishing the first carrier registry in Kelantan/Malaysia

Our PCR assay could match the culture results suggesting our PCR test could be an alternative method to detect for carriers

When screened for biomarkers, the carrier IgG and IgA showed that it recognized only the 50kDa antigen of *S.Typhi*. The *TYPHIDOT-C* was shown to be a potential serological screening tool to detect for possible carriers.

Established the first genome sequence bank of *S.Typhi* that are isolated from confirmed carriers from Malaysia (MyTyphoidgenom). The specific sites could not be considered of value as epidemiological markers as well as diagnostic purposes since they are no unique sequences for *S. Typhi* isolated from typhoid carriers. This revealed that typhoid carriage is NOT due to the organism but rather due to host factors.

Immunological profiling of the host showed that reduced levels of TH1 and TH2 cytokines might suggest a carrier status

The candidate gene (2136 bp Polynucleotide phosphorylase gene) was used to test if typhoid carrier state in humans was due to a mutation in this gene that was reported to determine virulence and carrier status of *S. Typhimurium* in mice. Results showed that there were 11 silent mutations in the PNPase gene (hence did not affect protein expression). Hence the findings reported for *S. Typhimurium* in mice cannot be extrapolated to *S. Typhi* that affect only humans

In developing the dipstick method for Antibody carrier detection, we have shown by POC that the dipstick method is working in our laboratory for detection of IgA. When outsourced for the cassette format for both IgG and IgA requires more time for fine tuning.